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# Factors Affecting Sertoli Cell Function in the Testis

## by D. J. Tindall,* J. S. Tash,* and A. R. Means*

**The Sertoli cell is the primary target for FSH action in the mammalian testis. These cells contain the majority of testicular plasma membrane receptors for this hormone. Receptor occupancy is directly correlated with a stimulation of adenylyl cyclase and a decrease in the activity of a cytoplasmic $Ca^{++}$-sensitive cAMP phosphodiesterase. Regulation of these two enzymes allows increased intracellular accumulation of cAMP, activation of cAMP-dependent protein kinase and phosphorylation of a variety of protein substrates. All of these events occur within the first 30 min following exposure of isolated Sertoli cells to FSH.**

**RNA and protein synthesis are also enhanced by FSH. Previous studies have suggested that this gonadotropin may augment the overall cellular synthesis of proteins. Our results reveal that protein kinase inhibitor (PKI) is selectively elevated by FSH both *in vivo* and *in vitro*. PKI thus becomes the initial intracellular protein whose synthesis is under FSH control. In addition to effects on protein synthesis, FSH also positively modulates the secretion of several specific proteins. One of the proteins in this latter category is androgen binding protein (ABP). Again, regulation can be observed both *in vivo* and *in vitro*. Elevated synthesis of PKI occurs prior to demonstrable secretion of ABP. Both of these events occur subsequent to the effects of FSH on cAMP metabolism. Indeed cAMP (or any of several nonhydrolyzable derivatives) can substitute for FSH *in vitro*. The temporal sequence of events subsequent to hormone binding and cAMP production are identical, but occur more rapidly. Together these data support the hypothesis that most of the biochemical steps leading to the synthesis and secretion of proteins by FSH are regulated by elevated levels of cAMP.**

## Introduction

The effects of toxic agents on the testis can be evaluated only by understanding the basic biological events which contribute to the normal functions within the testis. The Sertoli cell, to a large degree, dictates the development and maintenance of spermatogenesis within the testis. The Sertoli cell is regulated by both a steroid hormone, testosterone, and a peptide hormone, follicle stimulating hormone (FSH). Spermatogenesis is probably mediated by the action of these two hormones on Sertoli cells. By understanding the basic mechanisms by which hormones regulate the Sertoli cell and by identifying specific proteins which are themselves regulated by hormones we can begin to evaluate mechanisms by which toxic agents might be affecting spermatogenesis. Several recent reviews concern the hormonal regulation of spermatogenesis and the mechanism of action of FSH (*1-3*). In this article we will review three areas of FSH action: (a) The temporal sequence of events following addition of FSH to Sertoli cells in culture; (b) The stimulation of the synthesis of protein kinase inhibitor in the Sertoli cell; and (c) The hormonal regulation of androgen binding protein in Sertoli cells.

*Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

## Mechanism of FSH Action

A proposed sequence of events for FSH action in the Sertoli cell is presented in Figure 1. FSH binds to specific receptors on the membranes of Sertoli cells (*4-6*). This interaction is both tissue- and cell-specific, is dependent on both temperature and time, is of high affinity ($K_d = 10^{-10} M$), and is of limited capacity ($10^4$ sites per cell). Binding of [$^{125}$I]- or [$^3$H]FSH can be detected within 1 min, and saturation is achieved by 20 min. FSH-membrane binding coincides with stimulation of adenylyl cyclase (*3, 7, 8*). Cyclase stimulation can be observed either in

homogenates prepared from Sertoli cells, intact Sertoli cells incubated with [$\alpha^{32}$P]ATP, or in membranes isolated from the cultured Sertoli cells (*8*). In the latter case, a significant stimulation is noted within 2 min, and a linear increase in activity continues for at least 20 min. Thus, the temporal kinetics of the interaction of FSH with plasma membrane receptors and the activation of membrane bound adenylyl cyclase are similar.

Adenylyl cyclase is the only enzyme known to synthesize cyclic AMP (cAMP) in cells. Likewise, cyclic nucleotide-dependent phosphodiesterase is the only enzyme to degrade cAMP. A change in the $V_{max}$ of adenylyl cyclase is equal to an equivalent change in the $K_m$ of phosphodiesterase, which is inversely related to the $V_{max}$ of phosphodiesterase (*3*). Therefore, in considering the steady-state concentration of cAMP, activities of phosphodiesterase must be taken into account. Administration of FSH results in a decrease in the activity of cAMP phosphodiesterase in isolated Sertoli cells within 5 min; inhibition is maximal at 20 min (*3*, *4*, *9*). The temporal effects of FSH on phosphodiesterase are similar to FSH binding and stimulation of adenylyl cyclase. The phosphodiesterase isoform affected by FSH is a high affinity cAMP enzyme that requires calcium ($10^{-6}$ *M*) for activity (*3*). The stimulation of adenylyl cyclase and inhibition of phosphodiesterase result in an elevation of intracellular levels of cAMP (*10*). Significant effects can be seen at $10^{-11}$ *M* hormone, and maximal effects occur at $10^{-9}$ *M* (*4*). Thus over a two log dose range of FSH, a linear increase in Sertoli cell cAMP is observed.

The increase in the intracellular concentration of cAMP results in the activation of cAMP-dependent protein kinase (*2-4*, *8*, *11*). This activation can be seen within 2 min, is maximal within 30 min, and even in the continued presence of hormone declines to basal levels by 4 hr. The activated catalytic subunit of cAMP-dependent protein kinase phosphorylates proteins in virtually every subcellular compartment. Subsequent to the biological events described above, increased activity of RNA synthesis, protein synthesis and protein secretion are observed (*3*).

## FSH Stimulation of Protein Kinase Inhibitor

The heat-stable inhibitor of cAMP-dependent protein kinase (PKI) has been demonstrated to be under specific hormonal regulation by FSH *in vivo* (*12-15*). Significant increases in inhibitor activity in young animals were detectable as early as 8 hr after hormone administration, maximal levels (twofold

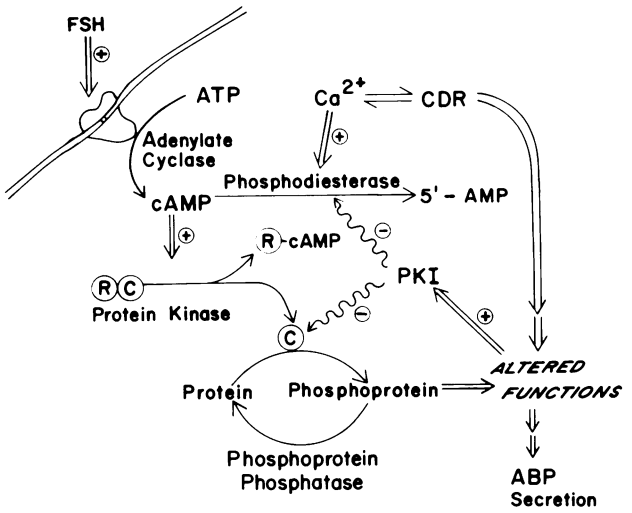


FIGURE 1. Proposed sequence of events for FSH action in the Sertoli cell.

increase) were achieved 24 hr after FSH treatment. Stimulation of PKI was specific to FSH; neither luteinizing hormone (LH) nor testosterone propionate could induce any change in testicular PKI activity. FSH stimulation of PKI was prevented if protein synthesis was blocked by administration of cycloheximide.

Sertoli cells cultured for 3 days in the continued presence of 5 μg FSH/ml (NIH-S-l2) and 5% rat serum contained significantly higher levels of PKI than cultures maintained in the absence of FSH (*12*). Culture conditions have been so modified that cells are maintained in completely defined medium in the absence of serum. Under these conditions in the absence of FSH, PKI activity in cells isolated from 16-day old cells drops markedly during the first 24 hr of culture. The low level of inhibitor activity reached after one day remains unchanged during the remainder of the 4-day culture period.

When Sertoli cells were cultured for 2-3 days in completely defined medium, it was found that the continued presence of FSH resulted in a 5- to 7-fold greater level of PKI activity than cells cultured for the same period of time in the absence of hormone (*14*). On the other hand, when cells were first cultured for 1 or 2 days without hormone, PKI activity declined. Subsequent exposure to FSH initiated a significant 3- to 4- fold stimulation in PKI activity (*13*).

The ability of FSH to maintain PKI activity in Sertoli cells (from 16-day old rats) was found to be dependent on the concentration of FSH (Fig. 2). Maximal PKI activity was observed in cultures containing 500 ng FSH/ml; half-maximal levels were achieved with as little as 80 ng FSH/ml (*14*).

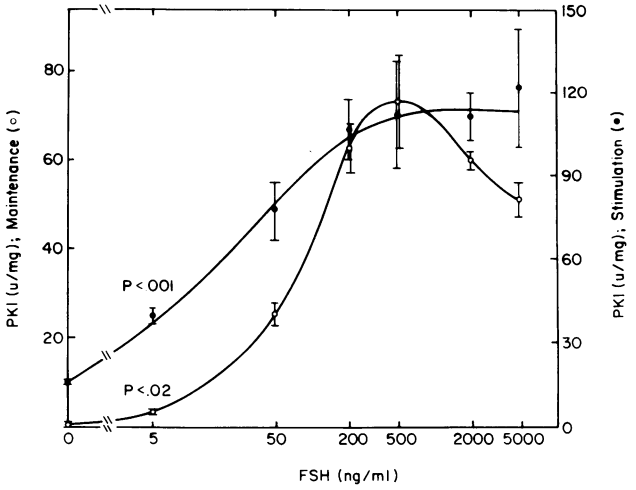


FIGURE 2. Dose-dependent maintenance and stimulation of PKI by FSH. Sertoli cells (from 18-day old animals) were cultured (O) for three days in the presence of FSH or (●) for two days in the absence of FSH followed by 24 hr in the presence of FSH (0 to 5 μg/ml). PKI activity was determined at each point in triplicate culture plates ± SEM (*15*).

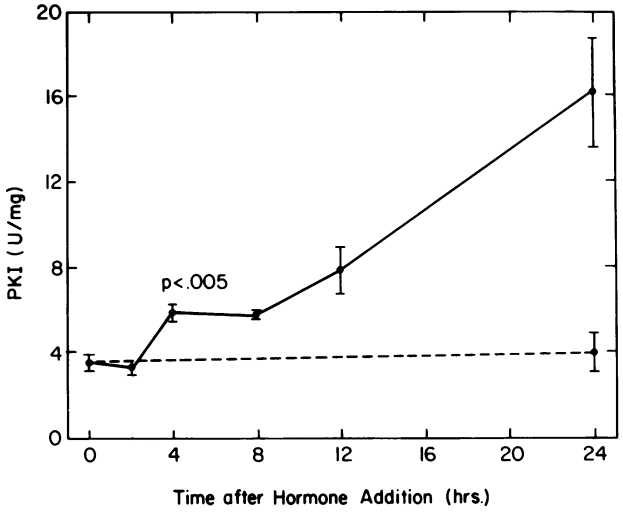


FIGURE 3. Stimulation of PKI by FSH *in vitro*. Sertoli cells were cultured for 48 hr in the absence of FSH, then for up to 24 hr in the presence of 500 ng/ml FSH (solid line). Control cultures (broken line) were maintained for the same period of time in the absence of FSH. Each point represents the mean ± SEM for 3-6 cultures (*14*).

The time course of PKI stimulation by FSH was next examined. Sertoli cells from 16-day old rats were cultured from two days without FSH, then for 24 hr with 500 ng/ml FSH. PKI activity was measured at 0, 2, 4, 8, 12, and 24 hr after hormone addition. A time-dependent increase in PKI levels occurred upon addition of FSH (Fig. 3). A significant increase in PKI activity (68%) was observed 4 hr after addition of FSH; by 24 hr PKI activity had increased 4-fold over control cultures not treated with FSH (*14*).

The stimulation by FSH of PKI in Sertoli cells from 16-day old rats can be mimicked by dibutyryl cAMP. A significant increase in PKI activity was observed only 2 hr after addition of dibutyryl cAMP ($1 \times 10^{-4}$ *M*) to the culture medium. Near maximal stimulation of inhibitor levels was obtained by 8 hr after dibutyryl cAMP addition (*14*).

The hormone specificity of the stimulation of Sertoli cell PKI was next examined. Cells from 16-day old animals were cultured for two days without hormone, then for 24 hr in the presence of FSH (500 ng/ml), LH (500 ng/ml), testosterone ($1 \times 10^{-6}$ *M*), or dibutryl cAMP ($1 \times 10^{-4}$ *M*). Of the hormones tested, only FSH induced a significant increase in PKI activity; it was also found that dibutyryl cAMP could mimic the effect of FSH (*14*).

These results demonstrate that protein kinase inhibitor in the Sertoli cell is regulated by FSH *in vitro*. These data corroborate previous results obtained *in vivo* (*12*) demonstrating that FSH specifically stimulates PKI activity in both normal and Sertoli cell-enriched testes from immature rats. In order to test whether the increased PKI activity resulted from increased synthesis, cycloheximide, a specific inhibitor of protein translation, was used. In the presence of cycloheximide alone no change in basal PKI activity was observed. Furthermore, no change in basal levels of protein synthesis as measured by incorporation of [$^3$H] leucine into TCA-precipitable material was observed. However, the addition of FSH to Sertoli cells containing cycloheximide failed to induce a response in PKI activity (*15*). These results suggest that the increased PKI activity found in the presence of FSH results from increased protein synthesis. The *in vitro* regulation of PKI synthesis was further evaluated *in vitro*. [$^{35}$S]methionine was added to cultured Sertoli cells and the incorporation of this labeled amino acid into protein kinase inhibitor was measured by precipitating the PKI with a monospecific PKI antibody. In the absence of dibuytryl cAMP, basal levels of [$^{35}$S]methionine incorporated into PKI precipitable material was very low. However, in the presence of dibutryl cAMP a 50-fold increase in [$^{35}$S]methionine incorporation was observed. In contrast, only a 23% increase incorporation of [$^{35}$S] into total TCA-precipitable was observed (*15*). These results suggest that FSH induces *de novo* synthesis of PKI in Sertoli cells.

## Hormonal Regulation of ABP

Another protein in the Sertoli cell which is stimulated by FSH is androgen binding protein (ABP). This protein is secreted by the Sertoli cell and transported through the efferent duct to the epididymis. It is thought to maintain high concentrations of androgen within the lumen by binding with high affinity. However, the biological function of ABP remains to be elucidated. Nonetheless ABP serves as an excellent marker for Sertoli cell function (*16*).

FSH was found to stimulate ABP in intact testes (*17, 18*), Sertoli cell-enriched rat testes (*19*), isolated seminiferous tubules (*20*), and Sertoli cells maintained in primary culture (*21, 22*). Although highly purified FSH elicited a response *in vivo*, there was no stimulation with thyroid stimulating hormone, prolactin or growth hormone. Luteinizing hormone stimulates ABP activity to varying degrees *in vivo*, however this may be due to its effects on testosterone synthesis (*19*). The response of ABP to FSH is related to the dose of FSH administered (*23*). Equivalent doses of different preparations of FSH gave similar dose-response curves in both the ABP assay and the ovarian weight augmentation test in hypophysectomized rats suggesting that FSH was the biological stimulus for both responses (*24*).

Testosterone will maintain spermatogenesis in the absence of FSH (*25*). Specific receptors for testosterone have been demonstrated in the Sertoli cell and may be involved in regulating the biological effects of this steroid on germ cells within the seminiferous tubules (*26*). However, until recently (*27*), there have been no documented responses of the Sertoli cell to testosterone. Testosterone will stimulate androgen binding protein activity *in vivo*. This has been demonstrated both acutely (*28*) and chronically (*29*). However, the acute stimulation appears to result from stabilization of the protein in the presence of ligand (*30*), whereas chronic treatment is more closely correlated with production of ABP. Chronic treatment of hypophysectomized rats with testosterone has demonstrated that this steroid will maintain normal levels of ABP in the apparent absence of FSH (*31*).

When testosterone is administered together with FSH *in vivo* an augmentation of the ABP response above that found with FSH alone is observed (*17*). This increased binding activity might be due to a synergism between testosterone and FSH. However, the increased binding may also have been due to the stabilizing influence of testosterone. In order to test these two possibilities, rats were injected for 4 days with either testosterone propionate, FSH or a combination of the two hormones (*23*). In rats treated with highly purified FSH for 4 days, no difference was found between these and the testosterone-treated rats. However, if the FSH treated testes were homogenized in the presence of stabilizing factors, a fourfold increase in binding activity was observed and no further change was seen in rats treated with FSH and testosterone propionate *in vivo*. These results demonstrated that testosterone, whether injected *in vivo* or included in the homogenization buffer, results in stabilization of any ABP present in the testis. Thus, the apparent synergism between testosterone and FSH is due to the stabilizing influence of the steroid on ABP binding activity. However, this does not rule out possible direct effects of testosterone *in vivo* on ABP production which were described previously.

There has been much confusion over which hormone, FSH or testosterone, regulates androgen binding protein. This has been complicated by a number of factors. These include the age of the animal, the length of time following hypophysectomy before hormone administration, and the dose of hormone. ABP secretion in immature rats less than three weeks of age, i.e., before lumen formation and secretion begins, has an absolute requirement for FSH (*32*). This cannot be replaced by testosterone. Even when secretion begins at about 16 days, administration of testosterone with FSH results in the abolishment of ABP secretion into the epididymis. This inhibitory effect of testosterone is not observed in rats 21 days or older. After 21 days of age, administration of testosterone propionate in large doses for 10 days will maintain levels of ABP similar to noninjected controls even though both FSH and LH are suppressed (*33*). Furthermore, FSH administration to older rats results in increased ABP binding activity in a dose-dependent manner (*23*).

Previous results suggested that changes in microfilament distribution could alter Sertoli cell shape and that cyclic AMP or low external calcium concentration (EGTA) could affect microfilaments in Sertoli cells (*8*). We then measured ABP secretion by isolated cells to see if EGTA would affect secretion. As shown in Table 1, EGTA caused rapid release of ABP, and cytochalasin B interfered with the EGTA effect. The results suggested involvement of microfilaments in the process of secretion of ABP.

The hormonal regulation of ABP secretion has been studied *in vitro* with testicular minces (*34*), explants (*35*) and Sertoli cells grown in culture (*2, 21, 22, 36*). However, these studies have not been as informative as initially hoped in that the specificity

Table 1. Sertoli cell secretion of ABP.[a]

| Treatment | Time, hr | ABP, pmole/ml |
|---|---|---|
| Control | 12 | 0.03 |
| FSH | 12 | 0.33 |
| EGTA | 1 | 0.28 |
| Cytochalasin B | 1 | 0.01 |
| EGTA + cytochalasin | 1 | 0.04 |

[a] Sertoli cells were isolated from Sertoli cell-enriched 16-day-old rats. Cells were incubated as duplicate samples in Eagle's minimal essential medium (containing no serum) at 37°C under $O_2/CO_2$ (95%/5%) in a shaker bath. Medium was assayed for ABP by steady-state polyacrylamide gel electrophoresis. FSH treatment was 10 μg/ml, EDTA was 4 *mM* and cytochalasin B was 10 μg/ml (*8*).

of the hormonal response is still unclear. Not only FSH but also TSH stimulates ABP *in vitro*. Moreover, testosterone appears to cause increased ABP secretion independently of FSH. Cortisol and 5α-androstane-3α,17β-diol resulted in significant elevation of ABP at early intervals following cell plating, but these responses disappeared with longer intervals of incubation. Even the presence of bovine-serum albumin caused elevated ABP activity *in vitro* as did whole serum from several species. Another problem with *in vitro* systems has been that the daily secretion rate of ABP gradually decreases even in the presence of hormones, although this is not evident if data are expressed as cumulative secretion. More studies are needed to evaluate the exact conditions necessary to maintain the integrity of the Sertoli cell and its secretory capacity *in vitro*. Once the proper environment for the Sertoli cell is established, one factor at a time can be removed in order to study its effect on secretory activity. Only then can we begin to fully understand the mechanisms by which ABP is regulated in the Sertoli cell.

## Summary

Results from our laboratory indicate that FSH is a primary regulator of Sertoli cell function. A variety of molecular events are observed following FSH administration to Sertoli cells in culture. Initially there is binding of FSH to plasma membrane receptors, followed by activation of adenylyl cyclase. This increases intracellular concentrations of cAMP which bind to the regulatory subunit of protein kinase causing release of the catalytic subunit. Protein kinase catalytic subunit phosphorylates specific proteins. These events are followed by increased protein synthesis and secretion by the Sertoli cell.

Protein kinase inhibitor activity is stimulated in the Sertoli cell in response to FSH. The increase in activity is due to FSH-regulation at the level of PKI synthesis. This protein will serve as a useful intracellular marker for FSH action in the Sertoli cell. Another protein which is regulated by FSH is the secretory protein, androgen binding protein. The activity of ABP is regulated not only by FSH, but also testosterone. Whether synthesis of this protein is regulated by both hormones remains to be determined. We have recently developed a monospecific antibody to ABP (*37*) and therefore should be able to examine this question by measuring incorporation of labeled amino acids into the protein.

Studies of the effects of toxic agents on the testis can be meaningful only by understanding the basic biological mechanisms involved in regulating spermatogenesis. We have elucidated some basic mechanisms by which hormones regulate the Sertoli cell. By using specific endpoint determinants of hormone action, such as protein kinase inhibitor and androgen binding protein, we can begin to examine the effects of toxic agents on spermatogenesis.

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